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LIGHT ENERGY CONVERSION IN HALOBACTERIUM HALOBIUM

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Halobacterium halobium carries out photophosphorylation. A rhodopsin-like protein, bacteriorhodopsin, located in the cell membrane mediates the first step in energy transduction, the conversion of light energy into a chemiosmotic gradient. After absorption of a photon, bacteriorhodopsin undergoes a series of fast reactions, returning to its original state in a few milliseconds. In continuous light it cycles continuously at 100 to 200 cps. During a cycle protons are taken up on the cytoplasmic side of the membrane and released on the outer surface, thus generating a chemiosmotic gradient which can drive phosphorylation of ADP to ATP.

Halobacterium halobium synthesizes ATP under anaerobic conditions in the light but not in the dark (1). The intracellular ATP concentrations maintained in the light are as high or higher than those found in the same cells under aerobic dark conditions. The light-induced ATP synthesis is sensitive to the uncouplers FCCP, CCCP, and DNP and to the ATPase inhibitors DCCD and Dio-9, but not sensitive to the electron transport inhibitors KCN, NQNO, DCMU, and antimycin. All of these agents inhibit ATP synthesis in cells respiring in the dark (1). Both respiring cells in the dark and anaerobic cells in the light eject protons into the medium. The acidification of the medium in respiring cells is abolished by uncouplers and electron transport chain inhibitors; in anaerobic cells in the light only uncouplers are effective. In respiring cells light inhibits respiration (2). These observations are easily understood if we assume with Mitchell (3) that ATP synthesis is driven by a chemiosmotic proton gradient across the cell membrane which can be generated either by respiration or by light and that uncouplers function as proton ionophores which dissipate the gradient. The observation that DCCD inhibits ATP synthesis but not acidification of the medium is strong evidence against the possibility that ATP synthesis is the primary event and that the gradient is generated at the expense of ATP.

Halobacterium halobium cells do not ferment carbohydrates and do not contain chlorophyll. Only cells grown at low oxygen tension are effective in light-induced ATP synthesis and proton ejection. Under these conditions the cells synthesize a rhodopsin-like protein, bacteriorhodopsin, which forms two-dimensional crystalline arrays in the

cell membrane, excluding all other membrane proteins from these patches (2, 4, 5). The patches can occupy more than 50% of the total surface area of the cell. In the visible spectrum bacteriorhodopsin shows one broad absorption maximum around 570 nm, giving it a deep purple color. We have therefore named these patches the purple membrane. Only cells containing purple membrane show light-induced ATP synthesis, inhibition of respiration, and acidification of the medium, and only light absorbed by the purple membrane is effective (1, 2). We may, therefore, assume that these effects are mediated by the purple membrane and that the light energy absorbed by the purple membrane is used to drive these processes, because there is no other apparent source of energy in anaerobic cells. The primary process appears to be the generation of the proton gradient, and we are thus led to the hypothesis that the purple membrane functions as a light-driven proton pump.

We have isolated the purple membrane (6, 7); it contains 25% lipid and 75% protein. Retinal is covalently bound to the protein in a molar ratio of 1. The linkage is a Schiff base between retinal and a lysine residue of the protein (4). Similar to the visual pigments, this retinylidene protein in its native form shows a large red shift of the absorption maximum from 370 nm to 570 nm, indicating further conformation-sensitive interaction between the protein and the chromophore. Unlike many visual pigments the purple membrane does not bleach significantly when it is exposed to light. Only a small shift in the absorption maximum, from 560 to 570 nm, is apparent (4, 8). It returns very slowly to the 560 nm maximum in the dark. The following observations all refer to the light-adapted 570 nm complex bR_{570}. Flash spectroscopy reveals that bacteriorhodopsin undergoes a fast cyclic photoreaction. Three intermediates have so far been tentatively identified spectroscopically (Fig. 1). The earliest change that can be observed with a time resolution of approximately 10 nsec is a shift of the absorption maximum to 610 nm. We call this the 610 nm complex, bK_{610}. It resembles prelumirhodopsin or prelumiiodopsin (9), because this shift occurs even at −196°C and it is photoreversible. The rise-time of bK_{610} has not been resolved so far. It decays in the dark at room temperature in $\sim$ 2 μsec to a new complex, bL_{550}, with an absorption maximum close to that of the original bacteriorhodopsin (bR_{570}). The next identified intermediate in the cycle, bM_{415}, is also formed in the dark, and at room temperature it shows a rise-time of $\sim$ 40 μsec and an absorption maximum at 415 nm. Under the same conditions it decays to the original bR_{570} complex in 5 to 10 msec. Thus, only the $bR_{570} \rightarrow bK_{610}$ transition requires light; all other reactions occur in the dark. The sequence of spectral changes is very similar to that observed in visual pigments except that bacteriorhodopsin returns to its original state in the dark and no additional energy is necessary to regenerate it. In strong continuous light of wavelength absorbed by the 570 nm complex, bacteriorhodopsin will therefore continuously repeat this reaction sequence at 100 to 200 cycles/sec at room temperature. The maximum rate of the cycling is temperature dependent and a $Q_{10} = 1.7$ has been determined for the $bK_{610} \rightarrow bL_{550}$ and the $bL_{550} \rightarrow bM_{415}$ transitions; the $bM_{415} \rightarrow bR_{570}$ transition has a $Q_{10} = 2.8$ for temperatures between 0° and 40°C (Fig. 2) (10, 11).

High concentrations of NaCl have little effect on the reaction cycle. If, however, a suspension of purple membrane in 4.0 M NaCl is saturated with ether, the $bM_{415} \rightarrow bR_{570}$ transition rate is slowed down and a half-time of 13 sec is now measured at room temperature. The other transitions are not significantly affected. In strong continuous light a steady state is therefore established in which most of the pigment is present in the form

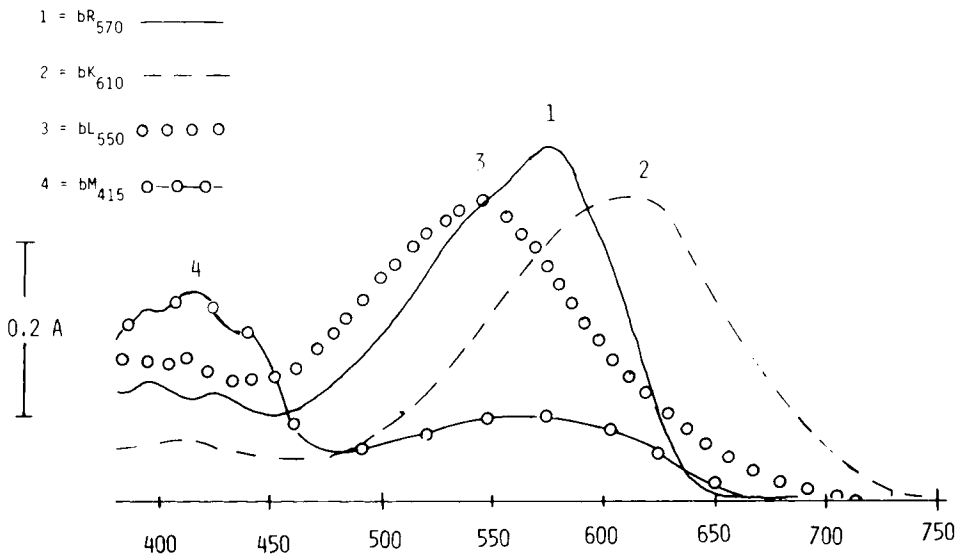

Fig. 1. Tentative absorption spectra at −196°C for the intermediates in the photochemical reaction cycle of bacteriorhodopsin which have been observed so far. Curves 1 and 4 were obtained by freezing light-adapted purple membrane in liquid nitrogen in the dark and in the light, respectively. Curve 2 was calculated from the spectrum after irradiating the dark frozen purple membrane at −196°C, and curve 3 was calculated from the spectrum obtained after transient warming of this preparation to −90°C.

of the bM_{415} complex. This allows observation of another photoreaction of the purple membrane. Measurement with a glass electrode shows that the pH of the membrane suspension is lower in the light than in the dark. The time response of the pH electrode is too slow to follow the change upon onset of the illumination; however, the return to the original pH upon cessation of illumination closely follows the spectral $bM_{415} \rightarrow bR_{570}$ transition. The purple membrane apparently releases protons when it goes through the first part of the photocycle and takes them up again in the $bM_{415} \rightarrow bR_{570}$ transition (12). At sufficiently high light intensities the bM_{415} complex is present in steady-state concentrations high enough to detect it spectrophotometrically, and the decrease in pH can be measured with a glass electrode even in the absence of ether. We conclude that the fast cyclic photoreactions are also accompanied by a release and uptake of protons.

These observations offer an explanation for the function of the purple membrane. If, during the rapid cycling of the pigment, protons were taken up and released on opposite sides of the membrane, the result would be a net translocation of protons across the membrane, provided the protein is uniformly oriented in the membrane. An asymmetry of the purple membrane is readily demonstrated by electron microscopy. Freeze-fracturing shows the cytoplasmic side of the fracture to be densely covered with particles which form a hexagonal lattice. Analogously to other membranes and model systems, we assume that these particles represent the protein (13, 14). The same hexagonal lattice seen in the electron micrographs has also been observed by x-ray diffraction in isolated purple membrane and cell envelopes (5). Preliminary evidence indicates that the protein may span the membrane (15). The morphological observations thus confirm the

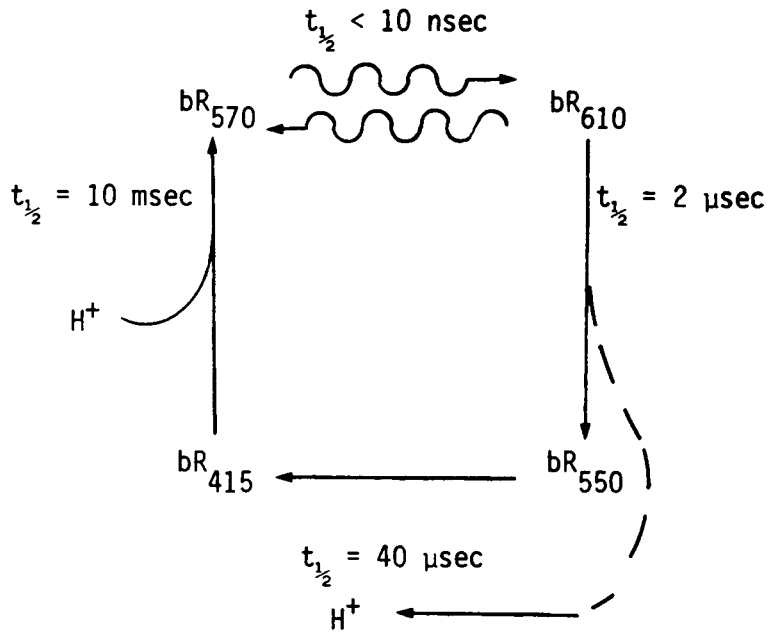

Fig. 2. Scheme for the photoreaction cycle of bacteriorhodopsin. Wavy arrows indicate light reactions and straight arrows indicate dark reactions. The subscripts indicate the approximate wavelengths for the absorption maxima of the intermediates. Half-times for each reaction are given for room temperature. The bR_{570} to bK_{610} reaction also occurs at liquid nitrogen temperature and is reversible. A proton is released somewhere between bR_{570} and bM_{415}, and another proton is taken up in the bM_{415} to bR_{570} transition. The number of identifiable intermediate complexes may actually be higher. Preliminary evidence indicates that another intermediate exists between bM_{415} and bR_{570}.

postulated asymmetric and uniform distribution of bacteriorhodopsin in the purple membrane.

The hypothesis that the purple membrane converts light energy into a chemiosmotic proton gradient and that the cell uses this energy to drive ATP synthesis and probably other energy-requiring processes is strongly supported by all experimental data from intact cells and isolated purple membrane. However, the energy metabolism of H. halobium is not fully understood, and other explanations for our observations are possible. It seems desirable, therefore, to obtain evidence for the postulated function of the purple membrane in a simpler system. We have developed a model system consisting only of phospholipid vesicles with incorporated purple membrane. Illumination in this system also generates a proton gradient across the vesicle wall. The sign of the gradient is reversed when compared to intact cells, and electron microscopy shows that the purple membrane is also preferentially oriented in the opposite direction (San-Bao Hwang and W. Stoeckenius, unpublished). The light-generated proton gradient in the model system can be used to drive synthesis of ATP from ADP and P_i if coupling factors and hydrophobic proteins from beef-heart mitochondria are incorporated into the lipid vesicles in addition to purple membrane. This model system carries out photophosphorylation (16).

Bacteriorhodopsin is held in a highly regular and apparently rigid lattice in the purple membrane. This is borne out by the high resolution of the x-ray diffraction

patterns obtained from wet purple membrane preparations (5), from spin label experiments (W. Hubbell and W. Stoeckenius, unpublished), and by flash spectroscopic studies of induced dichroism which indicate little if any movement of either the whole molecule or the chromophore during the photoreaction cycle (11). This makes it unlikely that the transport of protons across the membrane is based on rotation or translation of the whole bacteriorhodopsin molecule or parts of it. We rather think of a mechanism where absorption of a photon causes a pK change in a group in the protein, which is linked to one group on each surface through a proton transfer chain of suitable acidic and basic groups in the protein. The group changing its pK upon excitation could be the Schiff base between retinal and protein. It is not accessible from the medium in the bR_{570} complex but becomes accessible during the photoreaction cycle. It is protonated in the bR_{570} complex but unprotonated in the bM_{415} complex. The H^+ of the protonated Schiff base is not exchanged for a D^+ when the purple membrane is suspended in D_2O in the dark; however, upon illumination of the sample the H^+ is replaced by a D^+ (17). Finally, in isolated purple membrane the spectral shift accompanying the protonation in the $bM_{415} \rightarrow bR_{570}$ transition is insensitive to the pH of the suspending medium between pH 4.0 and 8.0, indicating that the proton appearing on the Schiff base is not directly derived from the medium (R. H. Lozier and W. Stoeckenius, unpublished). While these observations are compatible with the suggested model, they are far from sufficient to prove it. Variations of this and other models will have to be explored and many details will have to be filled in.

The purple membrane is apparently a light energy transducer which uses a mechanism quite different from the only other known biologic light energy transducers, the thylakoid membrane. Also, the pigment used is very different but closely resembles the pigments of the principal biologic light sensor, the eye, and the photoreactions too are very similar to those observed in the visual pigments. First, a very fast bathochromic shift occurs (prelumirhodopsin), followed by a shift back to a form with an absorption maximum close to the original pigment (lumirhodopsin) and then a large further blue shift (metarhodopsin). At this point, however, the analogy breaks down because bacteriorhodopsin returns spontaneously in the dark to the original form. This is, of course, essential for its function as an efficient light-driven proton pump. It is surprising to see that, in spite of the analogy to visual pigments, there is so far no indication of an isomerization of the chromophore in the cyclic photoreaction of bacteriorhodopsin (8, 18), while such an isomerization is thought to play an essential role in the photosensory function of the visual pigments (19).

Finally, bacteriorhodopsin functions not only as a light energy transducer but also as a light sensor (signal transducer). Bacteria containing the purple membrane show a phototactic response apparently mediated by the purple membrane (W. Stoeckenius, unpublished; 20), and the purple membrane may also mediate a light-induced permeability change in the rest of the surface membrane in H. halobium cells (21). Whether any of these functions are tied to the transition from the dark-adapted bR_{560} to the light-adapted bR_{570} complex, which is accompanied by a 13-cis to all-trans isomerization of the retinal (8, 18), remains to be seen; they could as well be secondary effects of the proton ejection.

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